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Malaria and intestinal helminthes co-infection and its outcome among febrile children suspected for malaria at Sanja Health Center, Northwest Ethiopia.

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LIST OF ABBREVIATIONS/ACRONYMS

BMI……Body Mass Index

ELPG……. Egg Load Per gram

Hgb……Hemoglobin

IL……Interleukin

ITBN…….Insecticide Treated Bed Net

STH……Soil Transmitted Helminth

Th…… T Helper Cell

TNF…….Tumor Necrosis Factor

WHO……..World Health Organization

ABSTRACT

Background: Malaria and helminthiasis are the two most epidemiologically co-existed infections and they cause for child co-morbidity in Africa. Few earlier studies in Ethiopia had also indicated co-morbidity of these infections. However, the rate of co-infection and related outcomes of malaria helminthiasis co-infection is not widely addressed in endemic countries.

Objective: To determine malaria, intestinal helminthes, co-infection and its outcome among febrile children suspected for malaria at Sanja Health Center, Northwest Ethiopia, 2015.

Method: A Cross-sectional study was conducted from February-April 2015 among febrile children at Sanja Health Center. Thick and thin blood films stained with Giemsa solution and slides were read under a light microscope using 100 × oil immersions. Similarly, Kato Katz concentration technique was used for confirmation and quantifying of ova in the stool. Data was analyzed by SPSS version 20 and software. Socio-demographic characteristics were summarized with descriptive tables. Bivariate, multivariate analysis was used to determine statistical association. $p<0.05$ was considered as statistically significant.

Result: A total of 357 study participants were participated, their mean age was 9.54 years. Among this 53% (190) were female. 70% were positive for malaria, *P.falciparum* was the leading followed by *P.vivax* 72.8%, 25.6% respectivey . 87.4% for intestinal helminthes and 62.18% co-infected by malaria intestinal helminthes. *S.mansoni* 56.3%, it is predominant, H.worm 12%, *A.lumbricoid* 10.6%, *H.nana* 4%, *E.vermicularis* 0.84%, and *Taenia species* 0.84%, *T.tricuria* 0.56%. Females were predominately affected by malaria and intestinal helminthes 54% (135), 53% (165) respectively. Malaria significantly associated with age, family size, insecticide treated bed net number and family income. Helminthes infection also associated with latrine, latrine usage. Anemia and malnutrition prevalence were high.

Conclusion: Malaria, intestinal helminthes and co-infection are major health problem in Sanja district. This finding showed that there is an association between malaria with age, Family size, insecticide treated bed net number and intestinal helminthes with latrine and latrine usage. Even though there was no statistical significant association, besides anemia and malnutrition were relatively high in co-infection.

Keywords: Malaria, intestinal helminthes, co-infection, nutrition, anemia Ethiopia**.**

1. INTRODUCTION

1.1. Background

Malaria is a protozoan disease of human caused by the genus *Plasmodium* with *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* as the main causative species. It is mainly transmitted to humans by the bite of infected female anopheles mosquitoes. Malaria due to *P. falciparum* is the most deadly form mainly in Africa while *P. vivax* is less dangerous but more widespread, and the other species are found less frequently [1]**.**

Malaria leads to severe and life-threatening disease characterized by a range of clinical features, including unrousable coma (cerebral malaria), severe anemia, metabolic acidosis, and multiorgan failure [2]. Pathogenic process of malarial anemia is multifactorial, even if it is only partly explained by the direct destruction of erythrocytes by parasites in the brain and other vital organs [3]. Several lines of experimental evidence showed that erythropoiesis can be severely disrupted by inflammatory mediators such as tumor necrosis factor (TNF). In vitro, TNF suppresses proliferation of erythroid progenitor cells in human marrow cultures [4].

On the other hand soil transmitted helminthes (STH) and schistosomiasis do not cause significant mortality compared to malaria, but cause substantial morbidity. Infections with STH have a pronounced impact on nutrition, growth, physical fitness, cognitive function and anemia mainly in children [5]. These infections have long term effects and are measured in terms of disease impact statistics, such as disability-adjusted life years [5, 6]. Helminthiasis has also a profound contrast effect on the immune system resulting in polarization towards T helper 2 (Th2) responses, characterized by high concentrations of cytokines such as interleukin-4 (IL-4), IL-5, IL-13 and high serum concentrations of immunoglobulin E (IgE) [6]. On the contrast effective immune response against malaria needs a strong inflammatory T helper 1 (Th1) response followed by the generation of a slowly developing protective antibody response. It is thought that the development of pathology in malaria infection is associated with the imbalance of cytokines involved in the regulation of inflammatory responses [7].

Mechanisms cause of anemia includes lysis and phagocytosis of infected red blood cells while the degree is de-pendent on the intensity of the malaria infection [8-10]. Helminthiasis on the other hand cause anemia from petechial haemorrhages such as direct blood sucking, which also leads to deprivation of nutrients and loss of appetite in the patient [11].

In the tropics, where malaria and intestinal helminthes epidemiologically co-existed, malaria–helminthes co-infection and co-morbidity is common. Concomitant parasitic infections could induce modifications of the specific immune response to each pathogen and clinical expression [12]. Studies found controversial outcome of malaria and helminthiasis co-infection [13-15].

Individuals are often co-infected with combinations of helminthes and malaria parasites [16, 17]. Co-infection may depend on spatial distribution of environmental conditions that favor the transmission of multiple species. In addition, immunological factors would be expected to influence the disease outcome of co-infection because helminthes modulate host immune responses both to themselves and to concurrent infections [18, 19].

In earlier studies of malaria and helminthiasis co-infection delayed malaria parasitaemia clearance and sever malaria found to be influenced with species and intensity of helminthes. Others had indicated the existence of opposite direction of outcome in the co-infection. In spite of this, anemia is the well described health related outcome by malaria and helminthiasis co-infection [20-23].

1.2. Statement of the problem

According to 2013 WHO malaria report, an estimated 3.4 billion people were at risk of malaria globally and populations living in sub-Saharan Africa have the highest risk of acquiring malaria. Approximately 80% of cases and 90% of deaths are estimated to occur in the African region. Malaria has been also recognized as the leading cause of infant morbidity and mortality in Africans [1, 24].

Malaria is one of the most widespread parasitic diseases all over the world [1] and half of the world's population lives in malaria-endemic areas with an estimated 500 million clinical cases and over one million deaths annually with majority of them from Africa countries [25]. It is estimated that over a third of the world's population, mainly in the tropics and sub-tropics, is infected with parasitic helminthes and *Plasmodium* species (*P. falciparum, P. malariae,P. vivax,* and *P. ovale)* , often leading to co-infections [26].

In Ethiopia, malaria is ranked as one of the leading communicable disease, accounting for about 30% of the overall disability adjusted life years lost [26]. Approximately 68% of the total population lives in areas at risk of malaria. In Ethiopia, *P. falciparum* and *P. vivax* are the major parasites accounting for roughly 60% and 40% of infections, respectively [1, 26, and 27].

Helminthic infections are also the most important causes of morbidity and mortality in many developing countries [28]. An estimated 1,471 million cases of infection with *Ascariasis*, 1,200 million cases of infection with hookworm infection, 1,049 million cases with trichuriasis, and 200–300 million cases with schistosomiasis occur worldwide [28, 29]. According to a World Bank report, morbidity due to helminthes infections accounts for an estimated 20% of the disability-adjusted life years lost due to infectious diseases in children less than 14 years old [30]. Helminthiasis due to hookworm infection, ascariasis, trichuriasis, and schistosomiasis are common tropical diseases with high endemicity in rural and in impoverished urban areas of low-income countries [34, 35].

Among the well-described morbidities associated with helminth infection in children are malnutrition, anemia, and failure to achieve their potential for growth [27, 31-32]. Previous study has shown that children presented with anemia and parasitic infections are more likely to be stunted and underweight than those who do not [33].

It is estimated that over a third of the world's population, mainly in the tropics and sub-tropics, is infected with parasitic helminthes and *Plasmodium* species often leading to co-infections [36]. Data on co-distribution of malaria and helminthes remains inadequate but in South East Asia and China, both *P.falciparum* and *P.vivax* appears overlap equally with all the three common STH [37].

Studies have shown that children having anemia as well as malaria helminthes co-infections are a number of times more likely to be stunted and underweight than those who do not. Nutritional problem and polyparasitism, out comes in malaria helminthes co-infection in children are more sever [38].

Malaria and helminthes can use different mechanisms to affect nutritional status of infected individuals [39-41]. Helminthes affect human gastrointestinal wall physiopathology, leading to intestinal inflammation, reduced appetite, mal-absorption and chronic blood loss [39, 40]. On the other hand, *Plasmodium* infection induces the secretion of inflammatory cytokines, which may in turn result in anorexia and catabolic responses, causing under nutrition in infected individuals [42]. Therefore, the effects of these parasites could be additive when they co-exist in a host. However, supporting evidences on malaria helminthes co-infection related outcome on nutritional status are limited [42].

However, the current status of malaria and helminthiasis co-infection and related outcomes are yet not widely understood in different malaria and helminthes co-endemic areas of Ethiopia. However, this cans intricate case definitions, treatments and prevention and control to be implemented in these areas. Therefore, this study is aimed to assess existing information about malaria-helminthes co-infection and related outcomes among febrile children suspected malaria at Sanja Health Center.

2. LITERATURE REVIEW

2.1. Prevalence of malaria among pediatrics children

Malaria as a cause of morbidity and mortality in children aged between 0–13 years in retrospective study conducted in Kenya. Prevalence of *Plasmodium falciparum* was lower among children with sickle cell disease (SCD) than among children without SCD 15.6%]. 16.3% of patients with SCD had features consistent with severe malaria, compared with 24.7% of patients without SCD [43].

Another study conducted in Kenya revealed that, Malaria and anemia prevalence among children was 10% to 34%, respectively. *P. falciparum* 92.3% was predominant and occurred in higher in children aged between 2–5 years. This study showed a positive correlation between the prevalence of anemia and malaria [44].

Additional cross-sectional study conducted in Kenya among children to assess the association between malaria and nutritional status revealed that 1.17% and 1.5%, low height-for-age and with low weight –for-age Z score, respectively. This study, suggests the absence of association between malaria and subsequent development of protein-energy malnutrition. However, age acted as an effect modifier in association between malaria episode and malnutrition [45].

A Cross-sectional survey conducted in Gabon. A total of 16,831 febrile children were enrolled; 78.5% (n=13,212) were less than five years old. The rate of *Plasmodium falciparum*-infection was the lowest in Port-gentil (below 10%) and the highest at Oyem (above 35%). Between 2005 and 2008, malaria prevalence dropped significantly from 31.2% to 18.3%, followed by an increase in 2011 in Libreville (24.1%), Port-Gentil (6.5%) and Oyem (44.2%) [46].

A cross sectional study conducted in Ghana 2001, among age groups in malaria, anemia (Hgb) and bed net usage, malaria parasite was found in 22% (515 of 2286) screened in May (dry-low transmission), and in 61% of the general population (1026 of 1676) screened in November (wet-high transmission). Malaria prevalence in adults was 54% (children 5–10 years). Age-specific malaria prevalence in November ranged from 38% (adults 50–60 years) to 82% (children 5–10 years). Prevalence of malaria parasite was significantly lower among young children whose

parents reported the use of bed nets. There was a significantly lower risk of malaria among young children living in the central, more urbanized sector of the study area [47].

A retrospective study conducted in Ethiopia, Kola Diba HC. Among 59, 208 blood films microscopically confirmed malaria cases were 39.6%. In this study, *Plasmodium falciparum* and *Plasmodium vivax* accounted for 75% and 25% respectively. Of the total malaria reported in all age groups and both sexes, the age group between 15–44 year and males were more affected [48].

2.2. Prevalence and related outcome of malaria helminthes co-infection

In a cross-sectional study conducted in Cameroon among malaria suspected children, the prevalence of malaria, intestinal helminthes and anemia was 50.7%, 22.3%, and 57.6%, respectively. In this study, the prevalence of malaria helminthiasis co-infection was 22.6%. [49].

Another study conducted in Bolifamba, Cameroon, among children revealed a prevalence of 64.2%, 38.3%, and 24.7% for *P. falciparum*, intestinal helminthes infections and *P. falciparum* and-helminths co-infection, respectively. Children co-infected with heavy helminthes intensity were diagnosed to with high *P. falciparum* than children con-infected with light helminthes intensity [50].

A cross-sectional parasite surveys conducted in Kenya, Uganda, and Ethiopia, Plasmodium–helminthes co infection are strongly influenced by the least common infection and by species-specific environmental factors. At the individual level, there is an enduring positive association between *P. falciparum* and hookworm but no association between *P. falciparum* and Schistosoma species. However, the relative importance of such within-individual associations is less than the role of spatial factors in influencing co-infection risks [51].

A cross sectional study conducted in Nigeria, a total of 178 (50%) enrollees were male. One hundred and fifteen of the 64.6% children had at least one intestinal helminthes infection while 60% there of harboured multiple helminthic infections. Malaria parasites were 19.7%) of the enrollees. Malaria-helminthes co-infection was detected in 20.9% (24/115) of the children. The

prevalence 52.2% (60/115) versus 12.7% (8/63) and severity of anemia were significantly higher among children with worms compared to those without worms. For mild anemia this was 46.8% (53/115) with worms versus 11.1% (7/63) for moderate anemia 1.74% (2/115); with worms versus 1.59% (1/63) without worms [52].

A cross sectional study conducted in Tanzania indicated a prevalence of *P. falciparum* malaria, *S. mansoni* and soil transmitted helminthes and any co-infections of *P. falciparum*, and intestinal helminths. 54.5% were infected with a single parasite species, 29% were infected with two or more species, and 16.5% had no infection. Prevalence of *P. falciparum* and *S. mansoni* were 13.5%, and 64.3% respectively. Prevalence of hookworm infection was 38%. *A. lumbricoides* and *T. trichiura* were not detected. Of the children 26.5% that harbored two parasite species, combination of *S. mansoni* and hookworm co-infections was 69%. Prevalence of *S. mansoni P.falciparum* co-infections was 22.6%, and that of hookworm *P.falciparum* co-infections 5.7%, Prevalence of co-infection of *P. falciparum, S. mansoni* and hookworm was 2.8% [53].

A cross-sectional study conducted in Tanzania, 69.8% children were infected with one or more parasites. Malaria helminthes co-infections were 60% of all children. Malaria parasites were significantly more prevalent in hookworm infected children than in hookworm free children. However, this association was non-significant on multivariate logistic regression analysis. Anemia prevalence was 34.4% and was significantly associated with malaria infection, with multiple parasite infections. *P. falciparum* infections were significant predictors of anemia [54].

A cross-sectional study conducted among malaria suspected febrile patients at Dore Bafeno HC, Southern Ethiopia,. 28.8% were positive for *P. parasites* (*P. falciparum* =13.0%, *P. vivax* =14.5%, mixed =1.3%). 53.8%, 31.6% and 19.4% were infected with intestinal helminths, Plasmodium alone and both Plasmodium and intestinal helminths, respectively. The prevalence of infections with *A. lumbricoides*, *T. trichiura*, *S. mansoni* and *H.worm* 9.8% were 35.9%, 15.8%, 11.7% and 9.8%, respectively. *P. falciparum* infection was more common in febrile patients infected with *A. lumbricoide* alone 21.3%, *T. trichiura* alone (23.1%) and *S. mansoni* alone (23.1%) compared to those without intestinal helminth infections (9.3%). Prevalence of non-severe malaria was significantly higher in individuals infected with intestinal helminths than

in those who were not infected with intestinal helminths. Non-severe *P. falciparum* malaria were 2.6, 2.8 and 3.3 times higher in individuals infected with *A. lumbricoides* alone, *T. trichiura* alone and *S. mansoni* alone, respectively, compared to intestinal helminth-free individuals [55].

A cros-cectional study conducted on Sidama zone south Ethiopia from 2010- 2011, among malaria suspected patients showed that 702 patients were seen, 34.5 %, 12.3%, 19.4%, 44.9% were infected with helminths alone, plasmodium alone, co-infected with plasmodium and helminthes, and undernourished respectively. The prevalence undernourished was not significantly different between those patients not infected with *plasmodium* or helminths species and those infected with plasmodium and helminths species [56].

A cross-sectional study conducted in Wondo Genet Health Center and Bussa Clinic, southern Ethiopia among malaria suspected patients revealed 67% and 53.1% of helminthiasis among malaria positive and malaria non-infected asymptomatic individuals, respectively. *Trichuris trichiura* infection was associated with increased malaria prevalence. Majority (77%) of the study subjects had multiple helminthic infections while the rate of individual helminths was 64.5, 57.7 %, 28.4%, and 12.2% for *T. trichiura, Ascaris lumbricoides, Schistosoma mansoni,* and hookworm, respectively [57].

Another cross sectional study conducted Alaba Kolito Health Center Ethiopia 2007 indicated, anemia has high significant association between malaria helminthes co infection in, among 458 febrile patients, Co-infection with *Plasmodium* and helminth parasites is associated with significantly higher anemia prevalence than single infection with *Plasmodium* parasites. And this difference was also significant for haemoglobin concentration (F = 10.18), in which patients co-infected with *Plasmodium* and helminthes parasites showed lower mean hemoglobin concentration. More than one-third of the infected cases in both malaria infections and malaria/helminthes co infections are undernourished [58].

A cross-sectional study conducted in Gondar Azezo Health Center Ethiopia among febrile patients the prevalence of malaria parasites were 11.5% (44) of which *Plasmodium vivax* and *P. falciparum* and mixed species infection accounted 75.0% (33), 20.5% (9), (4.5%) respectively.

Prevalence was higher in males (28) when compared infection (16). More than half (207, 53.9%) of study participants had one or more infection. Prevalence was slightly higher 52.7% (9109) than in males 47.3% (98). About helminthes, *Ascaris lumbricoides*, was the predominant isolate 62.1% followed by Hook worms 18.4%). Only 22 participants were co-infected with malaria parasite and helminthes and co-infected with *Ascaris lumbriciodes* was predominant 45.0%. The Prevalence of anemia was 10.9%. And co-infection with Plasmodium and helminthes parasites was significantly associated [59].

A study conducted in Gilgle Gibe Dam Ethiopia in 2010, malaria was 9.4%, in malaria positive cases W/A malnourished was 32.6%, in male and female were 35.4% & 32.9% respectively. Significant proportion (40.4%) of under-five children were stunted (height-for-age,-2 SD). The prevalence of under-weight was 34.2%. One third and one tenth of the children had anemia and malaria parasite respectively. Older children were more likely to have under-nutrition. There was no association between malaria and under-nutrition [60].

3. SIGNIFICANCE OF THE STUDY

According to WHO 2013 report, malaria is among the prior public health problem next to tuberculosis and HIV. Similarly; helminthiasis is one of the main causes of children developmental impairment.

Malaria and intestinal helminthes co-infection is among the great challenge of health problems in developing countries. This co-infection has synergetic effect and it increases treatment failures than single infection. Hence, the co-infection between malaria and helminthes could worse child morbidity.

Thus the current study describes the status of malaria and intestinal helminthes co-infection and the disease outcome among pediatric children in the study area. This will enable policy makers to look for better intervention methods where the diseases are endemic.

4. OBJECTIVE OF THE STUDY

4.1. General objective

To determine malaria intestinal helminthes co-infection and its outcomes among febrile children at Sanja Health Center Northwest Ethiopia.

4.2. Specific objectives

- To determine the prevalence of malaria among febrile children suspected for malaria at Sanja Health center, Northwest Ethiopia
- To determine the prevalence of intestinal helminthes among febrile children suspected for malaria at Sanja Health center, Northwest Ethiopia
- To determine the prevalence of malaria and intestinal helminthes co-infection among febrile children suspected for malaria at Sanja Health center, Northwest Ethiopia
- To determine outcome of malaria and intestinal helminthes co-infection

5. METHODS AND MATERIALS

5.1. Study area

The study was conducted at Sanja Health Center, Sanja, Tacharmachiho District, and Northwest Ethiopia. Sanja is located at 60 km away from Gondar and 807 km away from Addis Ababa, Ethiopia. Sanja has an altitude of 1800m above sea level; an annual rain fall of 800-1800 mm and annual temperature ranging from 25^{o}C to 42^{0}C. According to the 2005 Central Statistical Agency census in 2005, there was an estimated total population of 143,929 of which 70,585 are men and 73,344 are women, in the district. Of the total population, 6,799 or 4.72% of them are urban dwellers.

5.2. Study design and period

A cross-sectional study was conducted from February to April 2015 among febrile children suspected for malaria at Sanja Health Center.

5.3. Population

5.3.1. Source population

The source population was febrile children with clinical signs and symptoms consistent with malaria at Sanja Health Center.

5.3.2. Study population

Study population was febrile children with sign and symptoms consistent with malaria and attending Sanja Health Centre during the study period.

5.4. **Inclusion criteria**

Children with the age of 2-18 years

Children with clinical signs and symptoms consistent with malaria

Children attending the health center during the study period

Children voluntarily participates in the study

Children providing the required laboratory sample

Children diagnosed for malaria

5.5. **Exclusion criteria**

Pediatric children taking anti-malaria medication three weeks prior to the study commencement and during the study

Pediatric children taking anti-helminthes medication three weeks prior to study commencement and during the study

Sever ill children

5.6. Variables

5.6.1. Dependent Variables

Malaria- intestinal helminthes co-infection and its outcomes

5.6.2. Independent variables

- **Socio-demographic feature**
  - Age
  - Sex
  - Educational status
  - Residence
  - Monthly income
  - Occupation
- **Clinical feature**
  - Height, weight
- **Other risk factors**
  - Living around swampy area
  - Previous history of malaria infection
  - Bed net usage
  - Family size
  - Source of potable water
  - Hand washing habit
  - Wearing protective shoes
  - Latrine usage
  - Swimming habit
  - Middle upper arm circumference (MUAC)

5.6.3. **Operational and Standard definition**

Pediatric children: pediatric children mean that child with the age group of 2-18 years old [64].

Stunting: The term “stunting” is a condition in which children fail to gain sufficient height. It is an indicator of past growth failure [61].

Height-for-age (H/A): The term “stunting” is used to describe a condition in which children fail to gain sufficient height, given their age. Stunting is an extremely low “height- for-age” (H/A) score.

Weight-for-height (W/H): The term “wasting” refers to a situation where a child has failed to achieve sufficient weight for height (W/H). Weight-for height is normally used as an indicator of current nutritional status. Wasting may be the consequence of starvation or severe disease. It can also be due to chronic conditions or a combination of both.

Weight-for-age (W/A): The term “underweight” is used to describe a situation where a child weighs less than expected, given his or her age. Underweight is thus an extremely low “weight-for-age” (W/A) score. W/A reflects body mass relative to age. Unlike height, weight fluctuates over time and therefore reflects current and acute as well as chronic malnutrition. W/A is commonly used for monitoring growth and to assess changes in the magnitude of malnutrition over time.

Outcomes: a condition in which malaria suspected children manifest patient reported outcome (PRO) due to exposure of risk factor and children have clinical symptom upon clinical investigation with confirmation of laboratory diagnosis.

5.7. Sample size and sampling techniques

Sample size was estimated using single population proportion calculation. 67 % prevalence of malaria intestinal helminthes co-infection infection was considered [57]. Z = Standard normal distribution value at 95 % CI, which is 1.96; and 5% of marginal error was used.

$n = z^2\alpha/_2\, p\,(1-p)/w^2$ $\quad n= 1.96^2 * 0.67* (1-0.67)/0.05^2$

By taking Z= 1.96 at 95 % confidence interval

d= 0.05

p= 67 %

Considering 5% non response rate, the required sample size was 357.

5.8. Sampling technique

Study participants was selected with systematic random sampling technique and enrolled in to the study.

5.9. Data Collection and laboratory methods

5.9.1. Socio-demographic and clinical data

Questionnaire developed in English and translated to Amharic and translated back to English to maintain its consistency. Then, socio-demographic characteristics and clinical data were collected using interview-based questionnaire. The data was collected by trained Nurse/ HO. Then the patients sent to the laboratory to gave specimen for laboratory investigation.

5.9.2. Anthropometric measurements

The clinical data faced on the febrile children was collected using digital scale balance, scale empty weighing pants <5, the hook scale appropriately calibrate before we take the weight. For height measurement meter (measuring board for <5) on a hard flat surface against a wall, table, tree, staircase.

Calculate the midpoint of the child's left upper arm by first locating the tip of the child's shoulder with your finger tips. The most used indicators are stunting, wasting, and underweight, and mid-upper arm circumference in children under five years of age and Body Mass Index in adults. The recommended reporting system of H/A, W/H and W/A is in terms of Z- scores-a statistical measure of the distance from the median (mean) expressed as a proportion of the standard deviation by using WHO anthrop plus 2006 [61].

The most common cutoff point is –2 Z-score, i.e. two standard deviations below the median values of the international reference. This is the cutoff risk level used to differentiate malnourished children from those adequately nourished. Children whose H/A, W/H and W/A scores fall below this point are therefore considered, stunted, underweight and wasted, respectively. The WHO has proposed a classification scheme for population-level malnutrition [61].

5.9.3. **Laboratory sample collection and processing**

5.9.3.1. **Diagnosis of *Plasmodium* parasites**

Capillary blood was collected using sterile blood lancet, slides and disposable glove by experienced laboratory technologist after cleaning of the finger by sterile cotton ball soaked at 70 % alcohol. The first drop of blood was removed and the two consecutive drops were used for thin and thick blood film preparation and fourth blood for (Hgb) hemoglobin determination. The thin and thick smear was stained with Giemsa stain, and examined by two laboratory technologist independently for detection and identification of malaria parasite from all study participants [64].

5.9.3.2. **Diagnosis of intestinal helminthes**

The children who attend the Health Center seeking for clinical service were given clean and leak proof container with wooden applicator stick. After being informed on how collect stool sample, approximately 2 gram of fresh stool sample was collected from every study participant. Then the stool sample was processed using standardized template with Kato Katz technique which measures 41.7mg stool. The prepared stool was examined by two experienced laboratory technologist independently by using 10x objective of the microscope. Egg count for each species of the intestinal helminthes were recorded and converted to per gram (ELPG). Infection intensity for *S. mansoni* (1-99), (100-399) and (>400), *A. lumbricoides* (1-4999), (5000-49999), (≥50 000), *T. trichiura* (1-999), (1000-9999), (≥10000), *H. worm* (1-1999), (2000-3999), (≥4000) classified as light, moderate, and high respectively [62,64].

5.9.3.3. **Hemoglobin determination**

Hgb was determined by a portable hemoglobinometer (HemoCue, HemoCue HB 201 analyzer HemoCue ABS Kuvettgatan 1 SE-262 71 ÄNGELHOLM SWEDEN 2013) to determine anemic status. The Hgb level lower than 11.5g/dl was considered as anemic and 12g/dl and above are considered as normal (Hgb normal value is 12-18 g/dl) [63].

5.8.3. **Quality control**:

Pre-tested interview based questionnaire was used to collect socio-demographic data and was collected by trained data collectors. The reliability of study findings was guaranteed by implementing quality control measures throughout the whole process for example to check the performance by applying known positive and negative control slides. Double data entry system was used to maintain data entry quality. Any ambiguity appeared during data entry was re-checked from the original hard copy. Twenty percent (20 %) of Kato Katz and malaria blood films were re-checked by senior laboratory technologist for proper species identification determination of egg intensity of helminthes and malaria.

5.9. **Data analysis and interpretation:**

Raw data was entered in to EPI Info version 7 and then it was transferred in to SPSS version 20, WHO Anthroplus and WHO emergency nutrition assessment (ENA) 2006 for statistical analysis. Socio-demographic and clinical characteristics was summarized with descriptive table. Binary logistic regression and multivariate analysis were used to determine statistical association between dependent and independent variables. Odds ratio with 95 % confidence interval was used to measure strength of association between variables. Statistical significance was determined at 95 % CI with p-value less than 0.05. Results are presented in tables.

5.10. **Ethical Considerations**

Ethical clearance was reviewed and approved by the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar Research and Ethics Committee. Consultation and permission to conduct this study was also taken from Sanja District Health Bureau after explaining the benefit and risk of the study. After explaining the purpose, benefit and risk of the study, informed assent form and consent was taken voluntarily from children and child's parent/guardian, respectively.

Experienced Laboratory Technologist was taken three to four drops of blood under antiseptic techniques using sterile disposable gloves and disposable sterile lancets and 1-2 g of stool. Code, identification number was used instead of names for the confidentiality of laboratory results. Finally, Health Officer or Nurse in Sanja Health Center gave appropriate treatment to those children who were positive for malaria, intestinal helminthes and anemia according to the National Protocol. However treatment expense for malaria, helminthes and anemia cases was due by themselves according to the prescription since they are coming to the health center seeking for medical treatment.

6. RESULT

6.1. Socio demographic characteristics

A total of 387 study participants were participated in this study with the response rate of ninety five percent (95%). Their mean age was 9.54 years with standard deviation of 5.474. Among the participants 53% (190) and 47% (167) were females and males respectively. Most of the study subjects 65.3% (233) were urban residence, while 61% (218) of them were had illiterate family (Table 1).

Table1: Socio demographic characteristics of study subjects, at Sanja Health center, North West Ethiopia 2015.

Socio demography variables		Frequency(N)	Percent (%)
Sex	Female	N=190	53.2
	Male	N=167	46.8
Age	2-5	122	34.17
	6-11	90	25.21
	12-18	145	40.62
Residence	Urban	233	65.27
	Rural	124	34.7
Guardian	Government	33	9.24
occupation	Merchant	26	7.28
	Farmer	280	78.43
	Housewife	8	2.24
	Student	10	2.81
Family	Illiterate	218	61
education	Read and write	55	15.4
level	1^{st} cycle 1-8	37	10.36
	2^{nd} cycle 9-12	18	5
	College & above	29	8.1

6.2. Prevalence of malaria and intestinal helminthes and their co-infection

Auto of 357 participants 70% (250) were positive for plasmodium, of which 72.8% (182), 25.6%, 0.84% (3) (65) were infected with *P.falciparum, P.vivax* and mixed infections respectively. The overall malaria positive patients 54% (135) were females and 46% (115) were males, while in relation to age groups the distribution of malaria infection rate was similar.

Among a total of study participants 87.4% (312) were infected with intestinal helminthes, from which 53 % (165) were females. 2-5 and 12-18 years age groups were more exposed than 6-11 years for intestinal helminthes, which is 33.6% (105), 40.4% (126) and 25.9% (81) respectively.

In the current study the prevalence of *S.mansoni*, H.worm, *A.lumbricoid*, *T.trichiuria*, *H.nana*, *E.vermicularis*, and *Taenia* species were 83.5% (298), 12% (43), 10.8% (38), 0.84% (3), 3.9% (14), 0.84% (3), and 0.28% (1) respectively.

Clearly a total of 62.2% (222) study participants were found co-infected with both malaria and one or more than one intestinal parasites; of this co-infection malaria with *S.mansoni*, H.worm, *A.lumbricoides, H.nana were* 58.3% (208), 8.7% (31/357), 7.3% (26), and 3.1% (11) respectively. Among co-infections, 33.6% (120) were females and 28.6% (102) were males. From the overall co-infected participants 39% (139/357) were urban dwellers, while 41.5% (148/357) were Illiterates and 51.8% (185/357) from farmer families. Among co-infected study participants anemic prevalence was 5.3% (19/357) and participants having middle family size from 5-7 (32.8 % (117)) were more exposed, whereas children had nutritional problems (wasted and stunted) were 23% (82/357) and 31.6% (113/357) respectively (Table 2).

Table2: Prevalence of intestinal helminthes, malaria, and malaria intestinal helminthes co-infection among febrile children at Sanja Health Center, Northwest Ethiopia2015.

	Number (%) infected					
Intestinal parasite	Sex		Age group			Total
	Female	Male	2-5	6-11	12-18	
S.mansini	153 (42.9%)	145 (4%)	101 (28%)	76(21%)	121 (34%)	298 (84%)
H.worm	21 (5.9%)	22 (6%)	14 (3.9%)	10 (2.8%)	19 (5.3%)	43 (12%)
A.lumbricoide	21 (5.9%)	17 (4.7%)	15 (4.2%)	7(1%)	16 (4.5%)	38 (10.4%)
H.nana	10 (2.8%)	4 (1.1%)	3 (0.84%)	6(1.7%)	5 (1.4%)	14 (3.9%)
E.vermicularis	2 (0.5%)	1 (0.28%)	0 (0%)	2(0.5%)	1 (0.28%)	3 (0.84%)
Taenia species	1 (0.28%)	2 (0.5 %)	1 (0.28%)	1 (.28%)	1 (0.28%)	3 (0.84%)
T.tricuria	1 (0.28%)	1 (0.28%)	1 (0.28%)	1 (.28%)	0(0%)	2 (0.5%)
Malaria parasite						
P.falciparum	100 (28%)	82 (23%)	61(17%)	51 (14%)	70(20%)	182 (51%)
P.vivax	33 (9.2%)	31 (8.7%)	18 (5%)	20(5.6%)	26(7.3%)	64 (17.9%)
Mixed infection	1 (0.28%)	2 (0.5%)	2 (0.5%)	1(0.28%)	0 (0%)	3 (0.8%)
Co-infection						
Malaria and IH	120 (33.6%)	102 (28%)	70 (20%)	68 (19%)	84 (24%)	222 (62%)
Malaria/*S.mansini*	111 (31.1%)	97 (27%)	68 (19%)	61 (17 %)	79 (22%)	208 (56%)
Malaria/ *H.worm*	13 (3.6 %)	18 (5%)	11 (3.1%)	9 (2.5 %)	11 (3.1%)	31 (8.6%)
Malaria/*A.lumbricoide*	19 (5.3%)	12 (3.4%)	11 (3.1%)	9 (2.5%)	11 (3.1%)	31 (8.6%)
Malaria/ *H.nana*	2 (0.5%)	1 (.28%)	1 (.28%)	1 (0.28 %)	1 (.28%)	3 (0.84%)
Malaria/*E.vermicularis*	1 (0.28%)	0 (0%)	1 (0.28%)	0	0	1 (0.28%)
Malaria/*Taenia species*	1 (0.28 %)	1 (0.28 %)	1 (0.28%)	1 (0.28%)	0	2 (.5)

IH= intestinal helminth

From all febrile children, while those who are malaria positive only constitute 8.4% (30), intestinal helminthes positive alone were 4.2% (15). But, those who acquired intestinal parasite multiple infections with *S.mansoni*, *A.lumbricoides* and H.worm were 9.5% (34). Moreover, malaria positive children co-infected with *S.mansoni* and H.worm, were 0.6% (2) and *A.lumbricoides* and *H.nana* 2% (7).

When the frequency of heavy, moderate and light intensity of *S. mansoni* ova were 108, 112, and 78 respectively, Figure 1.

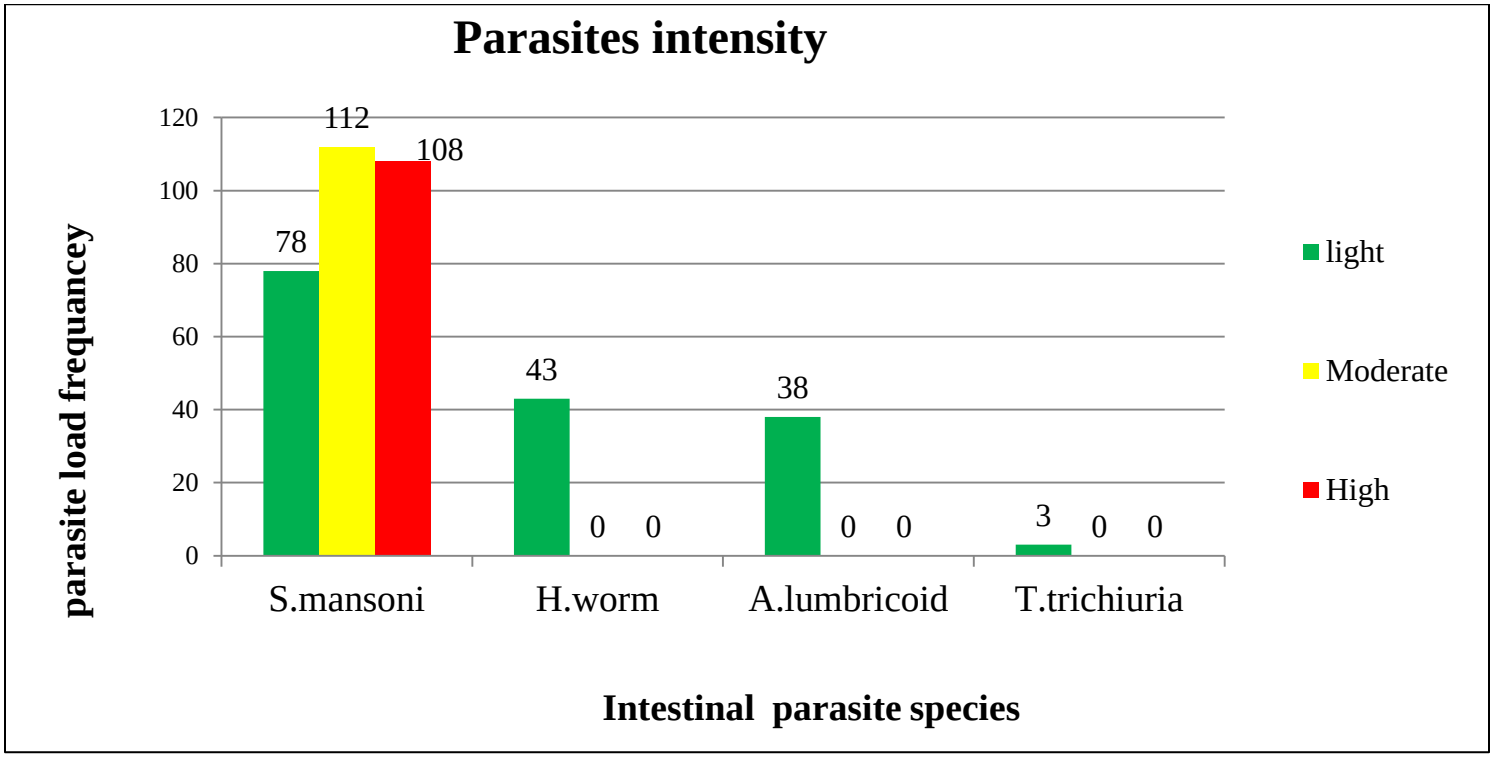

Figure1. Intensity of intestinal helminthes parasite load

6.3.**Prevalence of anemia in malaria intestinal helminthes and co-infection**

Auto of the total participants in the study period no child exposed to sever anemia, since all measured Hgb level revealed that ≥8 mg/dl Hgb in all sex and age groups recorded. Those to 2-5 6-11, 12-18 age groups 0.5% (2), 0.84% (3) and 2.5% (9), were moderately affected and 3.6% (13), 0.84% (3), and 0.84% (3) affected by mild anemia respectively. When from co-infected subjects 6% (21) were developing anemia, 8.1% (29) and 1.4% (5) anemic participants were affected by *S.mansonia and* Hookworm respectively. Moreover, among anemic participants 5.3% (19), 3.6% (13) and 2.5% (9) were stunted, malnourished, and wasted respectively.

While the prevalence of mild anemia in malaria infected study participants were 5.9% (21), P.falciparum contributes 75%. *S.mansoni* affection 8.4% (30), H.worm affected 1.4% (5), *A.lumbricoides* 3 (0.84%) and *H.nana* 0.56% (2) (Table 3).

Table 3. Prevalence of anemia by sex, age, malaria, Hgb concentration, intestinal helminthes and co-infection among febrile children suspected for malaria at Sanja Health center, Northwest Ethiopia, 2015.

Variables		Anemic N (%)	Non-anemic N (%)	Total N (%)
Sex	Female	16 (4.5)	174(48.7)	190 (53)
	Male	16 (4.5)	150 (43)	167 (47)
Age	2-5	10 (2.8)	112 (31)	122 (34.1)
	6-11	7 (2)	82 (23)	89 (24.9)
	12-18	15(4.2)	130 (36.4)	145 (40.6)
Malaria				
	P. falciparum	15(4.2%)	167 (47%)	182 (51.2%)
	P.vivax	5 (1.4%)	59 (16.5%)	64 (18%)
	Mixed	1 (0.28%)	2 (0.56%)	3 (0.84%)

Intestinal helminthes			
S.mansoni	30 (8.4%)	268 (75%)	298 (83.5%)
H.worm	5 (1.4%)	37(10.4%)	42 (11.8%)
A.lumbricoid	3 (0.84%)	35(9.8%)	38 (10.6%)
H.nana	2 (0.56%)	12 (3.5%)	14 (3.9%)
Co-infection			
Malaria/*S.mansoni*	19 (5.3%)	189 (52.9%)	209 (58.5%)
Malaria/H.worm	3 (.84%)	27 (7.8%)	31 (8.7%)
Malaria/*A.lumbricoides*	2 (.56%)	24 (6.7%)	26 (7.3%)
Malaria/*H.nana*	2 (0.56%)	9 (2.5%)	11 (3.1%)

6.4. Nutritional status among febrile children suspected for malaria

Among a total of 357 study subjects, 49.6 % (177) were stunted, 36.7% (131) were wasted and 30.3% (108) were malnourished (H/A, W/HA (BMIA), W/A SD +/–2 Z score below the median values of the international reference). From wasted study participants 21.5% (77), 8.6% (31), and 6.4% (23) were laid in age groups 2-5, 6-11 and 12-18 respectively. Majority of the age groups affected by wasted were <5 years. Among stunted children age group 11-18 was 29.4% (105). While in relation to sex the distribution of nutrition problem rate was similar. Even though, it does no reveal any statistical association with malaria, intestinal helminthes and co-infection.

Along with a total of nutrition problems caused by malaria parasites those who wasted, stunted, and malnourished were 26.4% (94), 36% (128), and 21% (76) respectively.

Intestinal helminthes also affected wasted 32.7% (177), stunted 43.3% (155), and malnourished 25.5% (91), while, co-infection causes 23% (82), 31.6% (113), 17.7% (62) respectively (Table 4).

Table 4. Nutritional status of malaria intestinal helminthes and co-infection among febrile children at Sanja HC, 2015. +/_2 SD Z scale in international reference

Variables		BMI (wasted) N%		HAZ (stunted) N%		WAZ (Malnourished)N%	
		Yes	No	Yes	No	Yes	No
		131(36.7)	226 (63.3)	177 (49.6)	168 (47)	108(30.3)	207 (58)
Sex	F	66 (18.4)	124 (35)	95 (26.6)	95 (26.6)	56 (15.7)	134 (38)
	M	65 (18)	102(28.9)	82 (30)	85 (23.8)	52 (14.6)	115 (32)
Age (years)	2-5	77 (21.5)	45 (12.6)	20 (5.6)	102 (28.6)	40 (11)	82 (23)
	6-11	31 (8.6)	59 (16.5)	52 (14.5)	38 (10.6)	25 (7)	65 (18)
	12-18	23 (6.4)	122 (34)	105 (29.4)	40 (11)	43 (12)	102 (29)
Malaria		94 (26.4)	156 (44)	128 (36)	229 (64)	76 (21)	281 (78.7)
Intestinal helminthes		117(32.7)	240(0.7)	155 (43.3)	202 (56.5)	91 (25.5)	266 (74.5)
Co-infection		82(23)	275 (77)	113 (31.6)	244 (68)	62 (17.7)	295 (82.6)
Anemia		9 (2.5)	348 (98)	19 (5.3)	338 (94.7)	13 (3.6)	344 (96.4)

6.5. Associated risk factors of plasmodium parasite among febrile children

During in this thesis data analysis, the effect of other confounding variables was controlled using multivariate logistic regression analysis to determine the dependent effect of each explanatory variable on the dependent variables. Risk factors associated with age groups 2-5, and 6-11 were associated with (p-value <0.05), educational level, family size, occupation, family monthly income and ITBN (insecticides treated bed net) number were significantly associated (P-value <0.05). Sex, residence, swampy area and ITBN were not associated with bivaraite and multivariate analysis results (Table 5).

Table 5. Bivariate and multivariate analysis of factors associated with malaria infection among febrile children suspected for malaria at Sanja Health center, North West Ethiopia 2015.

Risk factors	Malaria infection			OR (95%CI)		P-value
	Positive	Negative	Total	COR	AOR	
Sex						
Female	135 (38)	55 (15.4)	190 (53)			
Male	115 (32)	52 (14.6)	167 (46.8)	.767 (.451,1.305)	.833 (.468, 1.482,)	0.328
Age (Years)						
2-5	81(23)	41 (11.5)	122 (34.5)	1*		.033**
6-11	73 (10)	17 (4.8)	90 (25.2)	.174 (1.13, 4.15)	1.870 (.757, 4.61)	.019**
12-18	96 (27)	49 (13.7)	145 (40.7)	.992 (.596, 1.651)	1.141 (.514, 2.53)	0.974
Residence						
Urban	161 (45)	72 (20)	233 (65.3)	1*		
Rural	89 (25)	35 (9.8)	124 (34.7)	.889 (492, 1.605)	.880 (.456, 1.698)	0.696
Guardian educational level						
Illiterate	167 (46.7)	51 (14)	33 (9.2)	1*		.000**
Read and write	30 (8.4)	25 (7)	26 (7.2)	.366 (.198 , .679)	.489 (.225, 1.063)	.001**
1st cycle (1-8)	30 (8.4)	7 (1.9)	280 (78.4)	1.309 (.54 ,3.15)	.822 (.293, 2.306)	0.549
2nd (9-10)	9 (2.5)	9 (2.5)	10 (2.8)	.305 (.115 , .810)	.113 (.023, .549)	.017**
College & above	14 (3.9)	15 (4.2)	8 (2.2)	.285 (.129 , .630)	.134 (.024, .760)	.002**
Family sizes						
≤4	83 (23)	54 (15)	137 (38.4)	1*		.006**
5-7	132 (37)	38 (1.6)	170 (47.6)	2.260 (1.37, 3.71)	2.80 (1.397, 5.61)	.000**
≥8	35 (9.8)	15 (4)	50 (14)	1.518 (.757, 3.04)	1.514 (.567, 4.04)	0.239
Guardian/ Family Occupation						

Farmer	167 (47)	51 (14)	218 (61)	1*		0.187
Government employed	30 (8.4)	25 (7)	55 (15.4)	1.333 (.469, 3.79)	.388 (.078, 1.926)	0.59
house hold	30 (8.4)	7 (1.9)	37 (10.3)	2.278 (1.09, 4.74)	.382 (.072, 2.037)	.028**
Student	9 (2.5)	9 (2.5)	18 (5)	.833 (.1783,.911)	.170 (.020, 1.431)	0.817
Merchant	14 (3.9)	15 (4)	29 (8)	1.389 (.284, 6.79)	.452 (.044, 4.600)	0.685
Monthly income						
<500	28 (7.8)	2 (0.56)	30 (8.4)	1*		0.002
5001-1000	74 (20.7)	22(6.1)	96 (30)	.360 (.10, 1.299)	0.383(0.09,1.48)	0.002
1001-2500	88 (24.5)	37 (10)	125 (35)	.255 (.073, .890)	0.255(0.06,0.95)	0.003
>2500	60 (16.8)	45 (13)	105 (29.4)	.143 (.041, .500)	0.185(0.49,0.699)	0.037
Swampy area						
Yes	50 (14)	16 (4.5)	66 (18)	1*		0.999
No	200 (56)	91 (26)	291 (81)	.760 (.363, 1.594)	.829 (.381, 1.808)	0.468
Insecticide treated bed net(ITBN)						
Yes	220 (62)	95 (27)	315 (88)	1*		
No	30 (8.4)	12 (3.4)	42 (11.8)	1.970 (.28, 13.41)	1.417(.116, 17.35)	0.489
ITBN number						
0	25 (7)	11 (3)	36 (10)	1*		.005**
1	74 (21)	33(9)	107 (30)	1.679 (.2501, .27)	1.166 (.59, 2.305)	0.658
2	125 (35)	39 (11)	164 (45.9)	2.153 (.3241, .30)	.275 (.106, .712)	.008**
3	21 (5.88)	18 (5)	39 (10.9)	.485 (.0653, .645)	.199 (.029, 1.368)	0.101
4	2 (.5)	4 (1)	6 (1.67)	.342 (.025, 4.654)		

* Reference category, **significant association.

6.6. Association of intestinal parasite infection with risk factors among febrile children suspected for malaria

Intestinal helminthes infection with risk factors assessment revealed that no association with sex, age groups, family size, water source, swimming habit, hand wash, protective shoes wearing habit, educational status, and residence.

Regression logistic model showed in age group 12-18 1.031 times (COR= 1.031 95%CI0.718, 1.481) more likely infected than 6-11, 2-5 age group respectively. Being living in rural 0.860 times (COR=0.860 95% CI 0.450, 1.641) less likely infected with intestinal parasite. Having 5-7 family size 1.208 times (COR= 1.208 95% CI.616, 2.366) more likely infected with intestinal helminthes infection than ≤4 family size (Table 6).

Non-latrine users were 2.555 times (COR= 2.555 95% CI 1.322, 4.937) more likely affected by intestinal helminthes than latrine users, and those frequently washed their hands were less likely infected by intestinal helminthes.

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Table 6. Bivariate and multivariate analysis of factors associated with intestinal helminthes infection among febrile children suspected for malaria at Sanja Health center, North West Ethiopia 2015.

Risk factors	Intestinal helminthes			OR(95%CI)		P-value
	Positive	Negative	Total	COR	AOR	
Sex						
Female	165 (46)	25 (7)	190 (53.2)	1*		
Male	145 (41)	20 (5.6)	167 (46.8)	.114 (.594, 2.088)	.889 (.347, 2.275)	0.737
Age (Years)						
2-5	105 (29)	17 (4.7)	122 (34)	1*		
6-11	81 (22.7)	9 (2.5)	90 (25.2)	1.074 (.618, 3.438)	.970 (.214, 4.410)	0.39
12-18	126 (35)	19 (5.3)	145 (40.6)	1.031 (.718, 1.481)	.885 (.178, 4.415)	0.868
Residence						
Urban	205 (57)	28 (7.8)	233 (65.3)	1*		
Rural	107 (30)	17 (4.7)	124 (34.7)	0.860 (.450, 1.641)	.712 (.225, 2.256)	0.647
Guardian educational level						
Illiterate	189 (53)	29 (8.1)	218 (61)	1*		0.256
Read & write	51 (14.3)	4 (1.1)	55 (15.4)	1.956 (.658, 5.820)	5.121 (.765,34.294)	0.297
1st cycle(1-8)	32 (8.9)	5 (1.4)	37 (10.3)	.982 (.354, 2.724)	4.786 (.602,38.074)	0.664
2nd (9-10)	15 (4.2)	3 (0.84)	18 (5)	.767 (.209, 2.814)	11.854 (.147,99.62)	0.664
College and above	25 (7)	4 (1.1)	29 (8.1)	.959 (.311, 2.955)		
Family sizes						
≤4	118 (33)	19 (5.3)	137 (38.4)	1*		.
5-7	150 (42)	20 (5.6)	170 (47.6)	1.208 (.616, 2.366)	.998 (.323, 3.088)	0.583
≥8	44 (12.3)	6 (1.7)	50 (14)	1.181 (.443, 3. 149)	.929 (.187, 4.609)	0.74
Guardian/ Family Occupation						
Farmer	247 (69)	33 (9.2)	280(78.2)	1*		0.256
Government employed	27 (7.6)	6 (1.7)	33 (9.2)	1.663 (.639, 4.328)	4.829 (.061, 383.38)	0.999
Household	6 (1.7)	2 (0.56)	8 (2.2)	.667 (.107, 4.150)	25.183 (.3261, .946)	0.297
Student	2 (0.56)	0	2 (0.56)	.667 (.107, 4.150)	4.461 (.049, 410.06)	0.664
Merchant	24 (6.7)	2 (0.56)	26 (7.26)		2.474 (.024,250.45)	0.664
Monthly income						
<500	29 (8.1)	2 (0.56)	31 (8.7)	1*		0.707
5001-1000	83 (23.2)	13 (4.2)	96 (27)	.440 (.094, 2.070)		0.299

1001-2500	110 (31)	15 (4.2)	125 (35)	.506 (.109, 2.33)		0.383
>2500	90 (25.2)	15 (4.2)	105 (29.4)	.414 (.089, 1.918)		0.259
Source of water						
Tap water	233 (65)	38(10.6)	271 (75.9)	2.936 (.874, 9.865)		0.218
River	54 (16)	3 (0.8)	57 (16.8)	1*	4.925 (.506, 47.929)	0.082
Well	25 (7)	4 (1.1)	29 (8.1)	1.019 (.336,3.092)	.766 (.139, 4.207)	0.973
Swimming habit						
Yes	208 (58)	28 (7.8)	236 (66.1)	1*		
No	104 (29)	17 (4.8)	121 (33.8)	.824 (0.431, 1.573)	.101 (.001, 8.021)	0.556
Water exposure						
Always	62 (17)	5 (0.14)	67 (18.8)		1*	
Sometimes	147 (41)	24 (6.7)	171 (32.8)	2.024 (.739, 5.548)	.478 (.133, 1.709)	0.17
Protective shoes						
Present	294 (82)	44 (12)	338 (94.7)	1*		
Absent	18 (5)	1 (0.28)	19 (5.28)	2.694 (.351, 20.685)	.478 (.133, 1.709)	0.443
Hand wash habit						
Always	30 (8.4)	45 (13)	345 (21)	1*		
Sometimes	12 (3.4)	0	12 (3.4)	0.870 (.835, 0.906)	.632 (.094, 4.242)	0.341
Latrine						
Present	137 (38)	30 (8.4)	167 (46.8)	1*		
Absent	175 (44)	15 (4.2)	190 (53.2)	.391(.203, .756)		.005**
Latrine usage						
Always	97 (27)	17 (4.8)	114 (31.9)	1*		
Sometimes	45 (12.6)	13 (3.6)	58 (16.2)	.503 (.241, 1.053)	.999 (1.359, .000)	0.068
Not at all	170 (48)	15 (4.2)	185 (51.8)	.305 (.136, .688)	.999 (1.490, .000)	004*

* Reference category, **statistically significant association.

7. DISCUSSION

Globally, an estimated 3.4 billion people are at risk of malaria. WHO estimates that 207 million cases of malaria occurred annually [1]. Despite the fact that it is estimated over a third of the world's population, mainly in the tropics and sub-tropics, is infected with parasitic helminthes. Co-infection in a given community prerequisite for planning for combined intervention. In the current study, the prevalence of malaria was 70%. This result is very higher when compared to studied carried out Dore Bafeno Health Center, Southern Ethiopia 31.6% [55], Sidama zone, Southern Ethiopia 12.3% [56], Gondar Azezo Health Center (HC), Northwest Ethiopia 11.5% [59], Kenya range between 1.7% to 15.6% [43, 44, 45], Gabon 17.5%, Ghana 22%, Cameroon 50.7% [49], Nigeria 19.7% [52]. Perhaps this difference can be attributed from differences in genetics of subjects and immune responses, exposure to mosquito bites and existing malaria intervention measures.

Auto of the total malaria positive cases, the prevalence of *P. falciparum* confirmed in this study was 72.8%; in fact this is relatively lower than study conducted in Kenya 92.3% [44], on the other hand it is higher than studies conducted in Kenya 41.3% [45], in Bolifamba, Cameroon 64.2% [50], Dore Bafeno Health Center, Southern Ethiopia 13.0% [55], in Gondar Azezo Health Center northwest Ethiopia 20.5% [59].This is due to the fact that there may be climate and peak transmission season difference during the study period. Besides, this study finding result is comparable with Study conducted in Kola Duba Northwest Ethiopia 75% [48].

On the other hand the prevalence of *P.vivax* in this study was 25.6%, which is comparable to the study conducted in Kola Duba Northwest Ethiopia 25% and it is higher than by half, the study conducted in Dore Bafeno health center, Southern Ethiopia 14.5% [55]. Unlikely, this study is much lower than twice and as result showed that disagrees with study conducted in Gondar Azezo HC Northwest Ethiopia 75.0% [59]. This is may be due to time gap, climate, and geographical location.

As this study revealed that, there was 1.2% mixed malaria infection cases prevalence in this study, which is more or less comparable with a study conducted in Dore Bafeno HC, Southern Ethiopia 1.3% [55] and it is lower by half than a study conducted in Gondar Azezo Health Center Northwest Ethiopia 4.5% [59],

Obviously the intestinal helminthes prevalence assessment in this study revealed that 87.3%. This study result is very high compared to the study conducted in Cameroon 22.3% [49], in Bolifamba, Cameroon 38.3% [50], in Nigeria (64.6%) [52], in Tanzania 54.5% [53], in Tanzania 69.8% [54], in Dore Bafeno HC, Southern Ethiopia, 53.8% [55], in Sidama zone South Ethiopia 34.5% [56], in Wondo Genet HC and Bussa Clinic, southern Ethiopia 67% [57]. These differences may be due to sampled in different seasons, population socio-economic level difference, water resource, awareness towards intestinal helminthes transmission, parasite reservoir, fertile environment/ climate for specific helminthes hosts.

Eventually from this study each intestinal helminthes infection prevalence revealed that *S.mansoni was* 83.5% which is predominant in all of intestinal helminthes, this is very high when compare to studies conducted in Tanzania S. *mansoni* 64.3% [53], In Dore Bafeno HC, Southern Ethiopia, 11.7% [55], in Wondo Genet HC and Bussa Clinic, Southern Ethiopia 28.4% [57]. The main reason here may be, the Sanja River which is near to the Sanja town where it as good weather for the Snail reproduction and poor awareness of parasite transmission in the population, difference availability of tap water from place to place may vary with countries.

On the other hand, H.worm prevalence in this study was 12%, this is much lower than studies conducted in Tanzania 38% [53]. Reasonably this is may be due to time gap, sample size, method differences. This study results are comparable with studies conducted in Dore Bafeno HC, Southern Ethiopia, 9.8% [55], in Wondo Genet Health Center and Bussa Clinic, southern Ethiopia 12.2% [57].

Additionally *A.lumbricoides* in this study was 10.4%, probably this study finding was lower than studies conducted in Dore Bafeno HC, Southern Ethiopia, 35.9% [55], in Wondo Genet HC and Bussa Clinic, southern Ethiopia 57.7% [57]. This variation may be due to geographical location, and cultural behaviors of population.

Finally, *T.trichiuria* prevalence was 0.84% was not detected in Tanzania [53], this study finding was very lower than studies conducted in Dore Bafeno HC, Southern Ethiopia 15.8%, [55], in Wondo Genet HC and Bussa Clinic, southern Ethiopia 64.5% [57]. This is due to the fact that population culture awareness towards its transmission, climate, latrine usage habit, poor hygiene problem (consumption of shaded moist soil).

So far the study is concerned malaria intestinal helminthes co-infection showed that 62.2%, which is very high compared to studies conducted in Cameroon 22.6%. [49], in Bolifamba, Cameroon 24.7 % [50], in Nigeria 20.9% [52]. In Tanzania 2.8% [53], in Dore Bafeno HC, Southern Ethiopia, 19.4% [55], In Sidama zone south Ethiopia 19.4% [56], in Gondar Azezo Health Center Ethiopia 5.3% [59]. The main difference here is due to environmental or geographical factor associated with malaria and intestinal helminthes co-exition, reproduction place (soil), in fact there is Sanja River and Maho stream there, which is near to the town and they governs the nearby population throughout the year and specially which is the bet site for mosquito breeding, people water exposure awareness. Similarly, this study finding was in line with study conducted in Tanzania 60% [54].

As far as under the current study malaria affected cases showed that weight for age z scale (W/A) (malnourished) and height for age z scale (H/A) (stunted) children were 21% and 36% respectively. This is very high compared to study carried out in Kenya 1.17% and 1.5% [45]. This result attribution may be due to repeated malaria exposure, micronutrient provision, and other infectious diseases. On the other hand, this study was more or less comparable with study conducted in Sidama zone South Ethiopia 44.9% [56], in Gilgel Gibe Ethiopia 32.6% [60].

In the current study, there is no association between malnutrition problem with malaria, intestinal helminthes and co-infection cases, similarly, there was no any association observed study done in Gilgel Gibe Ethiopia. Prevalence of H/A +/-2SD Z scales in this study in male and female were 30%, 26.6% respectively, this result is relatively low when compare to study conducted in Gilgel Gibe Ethiopia 35.5%, 32.9% [60] in male and female respectively. More to the point, H/A in anemia was 2.5% in this study and it is in line with Gilgel Gibe Ethiopia 1.66% [60].

Besides that, the prevalence of anemia in this study was 9%, the result of this study was higher than study conducted in Nigeria 1.74% [52] and very lower than studies conducted in Cameroon

57.6% [49], in Tanzania 34.4% [54], in Kenya 34% [44], in Nigeria anemia in intestinal helminthes infected children 52.2%[52]. This difference might be due to the fact that, chronic and frequent infection of malaria and intestinal helminthes, micronutrient provision. On the other hand, this finding result comparable with study conducted in Kenya10% [43], in Gondar Azezo Health Center 10.9% [59]. Even though there was no association between anemia with malaria, intestinal helminthes and co-infection in this study. But there was an association between anemia and malaria study carried out in Kenya [44].

This study showed that, anemia increases as when age increases in malaria cases; while there was an association between age groups, family educational status, family occupation, family size, family monthly income, number of ITBN with malaria infection ($P<0.05$). in addition, there was an association between co-infection with age group ($P<0.05$). However, in this study intestinal helminthes infection revealed that, there was no association with socio demography characteristics and other associated risk factors except and the only associated risk factors were presence or absence of latrine and latrine usage.

8. LIMITATION OF THE STUDY

There were no additional hematological, organ function test and immunological laboratory investigation done in this study to see the effect of single or multiple parasite and host immune response and changes that occur during the presence and absence of specific parasites. Other factors like diet were not considered in this study. These things may have their own contribution to strengthen the finding in this study.

9. CONCLUSION

Malaria and intestinal helminthes co-infection is health problem among pediatrics children in Sanja district. This finding showed that there is an association between malaria with age, Family size, insecticide treated bed net number and intestinal helminthes with latrine and latrine usage. Even though there was no statistical significant association, besides outcomes of co-infection increased anemia and nutritional problems in the study participant.

10. RECOMMENDATION

Based on these study findings I recommend those things listed down

For government

- Insecticide treated bed net distribution shall be prioritized based on family size
- Safe water supply and latrine usage coverage shall be improved in the community
- Long term, sustainable, insured management implementation, control and monitoring plan should be applied to decrease malaria helminthes morbidity.

Health bureau

- Health education should be given to improve awareness towards infectious organisms.
- Continuous deworming is mandatory specially children.
- Malaria cases should be requested with stool examination and treated accordingly.

Researchers

- Further study needs to conduct in anemia and nutritional problems assessment.

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Annex I: Information sheet form for study subjects

Title of the proposal: malaria intestinal helminthes co-infection and its outcome among clinically malaria suspected children in Sanja Health Center Pediatrics OPD.

Name of the investigator: Habtie Tesaf

Purpose: to assess malaria intestinal helminthes co-infection and it's outcomes among malaria suspected children in Sanja Health Center Pediatrics OPD.

The investigator address: Department of Medical Parasitology School of Biomedical and Laboratory Sciences College of Medicine and Health S7ciences University of Gondar.

Procedures to be carried on: physical examination & laboratory investigations for malaria and other intestinal helminthes parasite infections were done.

Risks associated with the study: there is no risk particularly associated to this study.

Benefits of the study: the results of this study will be used as input for the planning of intervention activities for the prevention of malaria helminthes infections and treatment & care centers of the country.

Confidentiality of your information: the information obtained from the participants of the study will be maintained confidentiality.

Voluntary participation: participants of the study are not obliged to participate in the study. Therefore, participants are free to refuse from participation in the study any time if they are not willing to participate.

I would like to inform you that this study was approved & ethically cleared by School Ethical Clearance Committee, College of Medicine and Health Sciences, University of Gondar.

The addresses of the principal investigators are: habtietesfa@yahoo.com

Tell phone +251918732298

ስለመርምሩ አጭር መግለጫ

የምርምሩ ርዕስ፡ ወባ የአንጀት ጥገኛ ትላትል ጥምር እንፌክስን እና የሚያመጣዉ ችግር በህጻንት ላይ ስለማጥናት ይሆናል፡፡

የተመራማሪዉ ስም፡ ሀብቴ ተሰፋ

የተመራማሪዉ የሚሰራበት ተቋም ወይም አድራሻ፡ ጎንደር ዩኒቨርሲተይ ህክምናና ጤና ሳይንስ ኮሌጅ በባዮሜዲካል ት/ቤት እና ላቦራቶሪ ሳይንስ በፓራሳይቶሎጅ ትምህርት ክፍል፡፡

መግቢያ፡

የእርሰዎ ልጅ በዚህ ጥናት ዉስጥ በፈቃደኝነት እንዲሳተፍ ተጋብዟል፡፡

የጥናቱ አላማ፡

ልጅዎ በዚህ ትናት እንዲሳተፍ የተደረገበት ምክንያት ወባ እና የአንጀት ጥገኛ ትላትል በጥምር መበከል የሚመጣዉን ወይም ሚያስከትለዉን ተፅኖ ለማጥናት ነዉ፡፡

ዝርዝር ተግባር፡

ጠቅላላ የአካላዌ ምርመራ እና የላቦራቶሪ ምርመራ ጥናት ለወባ እና የአንጀት ጥገኛ ትላትል ምርመራ ይሰራል፡፡

የሚመጡ ተያያዥ ችግሮች፡

ልጅዎ በጥናቱ በመሳተፉ ምንም አይንት ተያያዥ ችግር የለም የሁን እንጅ ደም በሚወሰድበት ጊዜ መጠነኛ የሆነ የመርፌ ህመም ስሜት ሊኖር ይችላል፡፡

ጥናቱ የሚሰጠዉ ጠቀሜታ፤

የዚህ ጥናት ዉጤት እንደግብዓት ሊያገለግል የሚችልበት ብዙነዉ ለምሳሌ በሽታዎችን ለመቆጣጠረ ለሚደረጉ እንቅስቃሴወች ቅድመ ሁኔታወችን ለማመቻቸት በተለይ የሁለቱ ትገኛ በሽታ አማጭወች በአድላይ መገኘት ማለትም ወባና የአንጀት ጥገኛ ትላትል ለመከላከልና ለማከም በዓለምም የሁን በሀገር ዓቀፍ ደረጃ ከፍተኛ ትከሜታ ይኖረዋል ተብሎ ይታሰባል፡፡

የጥናቱ ተሳታፊ ካሳ፤

ማንኛዉም ተሳታፊ በጠጥናቱ ዉስጥ በመሳተፉ ምክንያት ምንም ዓይንት ክፍያ አይሰጠዉም፡፡

የተሳታፊዉን ሚስጥር ስለመጠበቅ፤

ማንኛዉም ተሳታፊ በጠጥናቱ ዉስጥ በመሳተፉ ምክንያት ምንም ዓይንት ግል ሰብዕናን የሚነካ መረጃ አይጠየቅም:: ነገር ግነ ጠናቱን በተመለከተ የሚወሰዱ መረጃወችን በተመለከተ ግን በሚስጠራዊነት ይተቢቃሉ::

በጥናቱ ሰለመቀጠል እና ሰለማቋረጥ፤

በጠጥናቱ ዉስጥ በመሳተፍ በፈቃደኝነት ተጠይቀዋል:: ከዚህባሻገር በጥናቱ መቀጠልም ያለመቀጠልም የሚስችል መብት አለወት::

የተመራማሪዉ አድርሻ:- ሀብቴ ተስፋ

የሞባይል ቁጥር 0918732298 ጎንደር ዩኒቨርሲቲ

Annex II: Consent form

I am conducting a study on the malaria intestinal helminthes co-infection and outcomes among malaria suspected children at Sanja Health Center. The purpose of this study is to determine the magnitude of malaria intestinal helminthes co-infections and associated outcomes among pediatric children. In this study, a drop of blood will be taken from your child for the diagnosis of malaria and determination of Hgb. In addition, a gram of fresh stool sample will be taken and analyzed with KKT for presence of intestinal helminthes parasite.

The result of this study will be used as in put for proper management of malaria and planning of intervention activities at malaria and helminthes co-endemic areas. There is no any compensation to be paid for your child being involved in this study. Information obtained from your child will be kept confidentially. You do have a right to declare that whether your child can participate or refused not to be involved in the study any time. I would also like to assure you that the protocol of this study is approved by the research and ethics committee of School of Biomedical and Laboratory Sciences. Above all participation of your child is very valuable for the fruitfulness of this study.

This consent form is read out to me in my own language. Therefore, with fulfill understanding of the situations. I agree my child to give the entire information, blood and stool sample voluntarily.

Participant secrete code number_____ Date ____ / ___ / ___

Family/ Guardian Name _____Signature _____ Date ____ / ___ /___

Witness Name _____ Signature _____ Date ____ / ___ / ___

Investigator Name _____ Signature ____ Date ____ / ___ / __

<u>የስምምነት ማረጋገጫ ፊርማዎች፤</u>

የወባ የአንጀት ጥገኛ ትላትል ጥምር እንፌክሽን እና የደም ማነስ ያለባቸዉን ሕጻናት ላይ ያለዉን ተያያዥነት ችግር ለማጥናት ሲሆን ጥናቱ የሚካሄደዉ በሰሜን ምዕራብ ኢትዮጵያ በሳንጃ ጤና ጣቢያ ነዉ፡፡ የትናቱ አላማ የወባ የአንጀት ጥገኛ ትላትል ጥምር እንፌክሽን እና የደም ማነስ ያለባቸዉን ሕጻናት ላይ ያለዉን ተያያዥነት ችግር ለማጥናት ነዉ፤ በዚህ ጥናት ጠብታ ደም በመዉሰድ ለወባ ምርመራና እና ሄሞግሎቢን መጠን መለካት ሲሆን በተጨማሪ ትንሽ ዓይነምድር በመዉሰድ የአንጀት ጥገኛ ትላትልን መኖር አለመኖሩን ለመመርመር ነዉ፡፡

የዚህ ጥናት ዉጤት ወባን ለመከላከልና የአንጀት ጥገኛ ትላትል እንፌክሽን በህጻናት ላይ ለመቀነስ ያገለግላል፤ ልጅወ በጥናቱ በመሳተፉ ምንም አይነት ክፍያ አይፈጸምበትም፤ ከልጅዎት የተገኘዉ መረጃ ከጥናቱ ዉጭ ለምንም አይነት አገልግሎት አይዉልም፤ ልጅወ ከጥናቱ በማንኛዉም ሰዓት ጥናቱን ማቋረጥ ይችላል፤ ይህ ጥናት በባዮሜዲካል ላቦራቶሪ ሳይንስ ት/ቤት ህጋዊነቱ የተረጋገጠ ነዉ፤ ስለዚህ ለልጅዎት ጥሩ የትናት ዉጤት እንዲሆን እመኛለሁ፡፡

እኔ ከዚህ በታች ስሜ የተገለጸዉ በዚህ ጥናት ልጄ ተሳታፊ በመሆኑ ደምና ሰገራ እንዲሰጥ ስወስን የጥናቱ አላማወች አሰራሮችና ቅድመ ሁኔታወች በግልጽ በመረዳት ፈቃደኝነቴን በማረጋገጥ ነዉ፡፡

የተሳታፊዉ የሚስጥር ቁጥር _____ ቀን --------

የተሳታፊዉ ;/የወላጅ/ አሳዳጊ ሙሉስም _____ ፊረማ _____ቀን _____

የተመራማሪዉ ሙሉ ስም _____ ፊረማ _____ ቀን --------

የተመራማሪዉ አድራሻ፡-

ጎንደር ዩኒቨርሲቲ የሞባይል ቁጥር 251918732298

የተመራማሪዉ አማካሪዎች አድራሻ፡-

ጎንደር ዩኒቨርሲቲ ሲሳይ ጌጤ የሞባይል ቁጥር 251910082887

ጎንደር ዩኒቨርሲቲ መሠረት ብርሃኔ የሞባይል ቁጥር 251918735636

ጎንደር ዩኒቨርሲቲ ዶ/ር ሙሉጌታ አዕምሮ የሞባይል ቁጥር 25113073815

በመጨረሻም ስለጊዜዎ አመሰግናለሁ፡፡

Annex III: Questionnaires

Department of Medical Parasitology Post Graduate Program School of Biomedical and Laboratory Sciences College of Medicine and Health Sciences University of Gondar

This semi-Structured questionnaire interview prepared to assess the socio demographic characteristics and outcomes of symptomatic malaria intestinal helminthes co-infection and its outcome among pediatrics children in sanja health center. Habtie Tesfa is student of University of Gondar in postgraduate programme in medical parasitology. Currently I am doing masters in titled with malaria helminthes co-infection and its outcomes among malaria suspected children attending at pediatrics OPD in Sanja Health Center, North West Ethiopia.

Part I- Socio demographic characteristics for malaria intestinal helminthes co-infection patients.

Patient code_____ (In figure)

1. Age (mm/dd/ yy) _____/ _____/ _____ (in figure) = _____
2. Sex A. Male B. Female
3. Place of residence A. Urban B. Rural
4. Guardian occupation A. Farmer B. Government employed C. Unemployed D. Housewife E. Student F. Merchant G. others specify _____
5. Family educational level A. Illiterate B. Read and write C. Primary education (1-8) D. Secondary (9-12) E. College and above.
6. Is there any pond water or swampy area in your environment? A, Yes B, No
7. Do you have insecticide treated bed nets? A, Yes B, No
8. Number of insecticide treated bed nets in a family_____
9. If your answer question number 7 “Yes” how often you use insecticide treated bed nets? A. always B. sometimes C. Not at all
10. Family size _____
11. Family monthly income A. <500 Birr B. 501-1000 C. 1001-2000 D. >2000

Part II. Clinical data

1. Is there fever in the past 24 hours? A. yes B. No "if yes" temperature _____(in figure)
2. Is there anemia A. yes B. No
3. Have you ever sick of malaria? A. Yes B. No
4. If your answer for question number 3"yes" is How many times? A. 1-2 B. 3-5 C. >5
5. Did you get treatment for these malaria episodes? A. yes B. No
6. Weight of the children, In figure _____
7. Height of the children, In figure _____
8. MUAC of the children, In figure _____

Part III. Question related with associated risk factor with intestinal helminthes infection.

1. What is your source of water for drinking, cooking and washing?
   A. River B. tap water C. well D. others specify
2. Do you have habit of swimming? A. yes B. No
3. How often you swim? In figure _____
4. Do you use latrine? A. yes B. No
5. If your answer is" yes" for question no 4, how often you use latrine? A. always B. some times
6. If your answer is no for question no 4, where do you defecate and dispose the faeces?
   A. Near to the river B. Open field ... Others specify _____
7. Do you use protective shoes? A. yes B. No
8. If your answer is "yes" for question no 7, how often? A. always B. some times
9. Do you wash your hand before eating food? A. yes B. No
10. If your answer is "yes" for question no 9, how often? A. always B. some times

Names of data collector _____ checked by _____

Sign _____ sign _____

Date _____/ _____/ _____ Date _____/ ___/ ___

አማረኛ ቃለ መጠይቅ፤

በፓራሳይቶሎጅ ት/ክፍል የሁለተኛ ድግሪ ፕሮግራም በባዮሜዲካል እና ላቦራቶሪ ሳይንስ ት/ቤት ህክምናና ጤናሳንስ ሳይንስ ኮሌጅ ጎንደር ዩኒቨርሲቲ፤

ይህ አማረኛ መጠቅ የተዘጋጁ የወባ የአንጀት ጥገኛ ትላትል ጥምር እንፌክሽን እና ሕጻናት ላይ ያለዉን ተያያዥነት ችግር ለማጥናት ሲሆን፤ ጥናቱን የሚያኪያሂደዉም የጎንደር ዩኒቨርሲቲ የሁልትገኛ ድግሪ ተማሪ የሆነዉ አቶ ሀብቴ ተስፋ ሲሆን የሚያጠናዉ ርእስም የወባ የአንጀት ጥገኛ ትላትል ጥምር እንፌክሽን እና የሚያስከትለዉ ችግር የወባ ምልክት በሚያሳዩ ሕጻናት ላይ ነዉ፡፡ ጥናቱ የሚካሄደዉ በሰሜን ምዕራብ ኢትዮጵያ በሳነጃ ጤና ጣቢያ በሕጻናት ሕክምና ክፍል ከሚከታተሉ የወባ ምልክት ከሚያሱ ሕጻናት ላይ ነዉ፡፡

<u>ክፍል 1፡- ማህበራዊና አካባቢያዊ ጥናትን በተመለከተ፤</u>

የተሳታፊዉ መለያ ቁጥር _____

1. ዕድሜ (ወር/ቀን/ ዓመት) _____/ _____/ _____ ዓመት_____(በቁጥር)
2. ፆታ ሀ. ሴት ለ. ወንድ
3. መኖሪያ ቦታ ሀ. ከተማ ለ. ገጠር
4. የቤተሰብ የስራ ሁኔታ ሀ. ገበሬ ለ. የመንግስት ሰራተኛ መ. ስራ የሌለዉ ሐ. የቤት እመቤት ሠ. ተማሪ ረ. ነጋዴ
5. የቤተሰብ የትምህርት ደረጃ ሀ. ያልተማረ ለ. ሐ. ማንበብና መፃፍ የሚችል መ. የመጀመሪያ ደረጃ (1-8) ሠ. ሁለተኛ ደረጃ ያጠናቀቀ (9-12) ረ. ኮሌጅና ከዚያ በላይ
6. የተቋረ ዉሃ በመኖሪያ አካባቢዉ አለ? ሀ. አዎ ለ. የለም
7. በቤት ዉስጥ በኬሚካል የተነከረ የአጎበር አለ? ሀ. አዎ ለ. የለም
8. በቤት ዉስጥ በኬሚካል የተነከረ የአጎበር ብዛት_____
9. አዎ ከሆነ መልስወት ቁጥር 7 የተነከረ የአልጋ አጎበር ለምን ያክል ጊዜ ይጠቀማሉ? ሀ. አልፎ አልፎ ለ. ሁል ጊዜ
10. የቤተሰብ አባላት ብዛት_____
11. ወረሃዊ የቤተሰብ ገቢ ሀ. ከ 500ብር ያነሰ ለ. ከ501-1000 C. ከ1001-2500 D. ከ2500 በላይ

ክፍል 2፡- ክልኒካል ሪከርድና ጠቅላላ የደም ሕዋሳት ምረመራ

1. በሀያ አራት ሰዓት ዉስጥ የሰዉነት የሙቀት መጠን ጨምሮ ነበር? ሀ. አዎ ለ. የለም
2. የደም ማነስ ምልክት ሀ. አለ ለ. የለም
3. ወባ አሞህ ያዉቃል? ሀ. አዎ ለ. የለም
4. ጥያቄ ቁጥር 3 አዎ ከሆነ መልስዎት ለምን ያክል ጊዜ? ሀ. 1-2 ለ. 3-5 መ. >5
5. በታመሙበት ጊዜ ህክምና አግንተህ/ሽ ታዉቃለህ/ሽ? ሀ. አዎ ለ. የለም

6. ክብደት _____ኪ. ግራም
7. ቁመት _____ሳ.ሜትር
8. የክንድ ዙሪያ ልኬታ _____ሳ.ሜትር

ክፍል 3፤ የአንጀት ጥገኛ ትላትል ተያያዥነት ያላቸዉ መጠይቅ

1. ለምግብ፣ ለመጠጥ፤ ለማብሰያ የሚዉል ዉሃ ከየት ነዉ የምታገኙ? ሀ. ወንዝ ለ. ቧንቧ ዉሃ መ. ጉድጓድ ሠ. ሌላም ካለ _____
2. ዉሃ ዋኝተህ/ሽ ታዉቃለህ/ሽ? ሀ. አዎ ለ. የለም
3. አዎ ከሆነ መልስወት ለምን ጊዜ? ሀ. አልፎአልፎ ለ. ሁልጊዜ
4. ሽንት ቤት ትጠቀማላችሁ? ሀ. አዎ ለ. የለም
5. አዎ ከሆነ መልስወት ጥያቄ ቁጥር 4 ለምያከል ጊዜ? ሀ. አልፎአልፎ ለ. ሁልጊዜ
6. አይደለም ከሆነ መልስወት ጥያቄ ቁጥር 4 የት ይጠቀማሉ? ሀ. ሜዳላይ ለ. ወንዝ አጠገብ ሌላ ካለ ይጥቀሱ_____
7. ናሙና የሚሰጠዉ / ምትሰጠዉ ህጻን ጫማ ለብሳለች/ለብሷል? ሀ. አዎ ለ. የለም
8. አዎ ከሆነ ጥያቄ ቁጥር 7 ለምንያክል ጊዜ? ሀ. ሁል ጊዜ ለ. አልፎአልፎ
9. ከምግብ በፊት እጅህን/ሺ ትታጠባለህ/ሺ? ሀ. አዎ ለ. የለም
10. አዎ ከሆነ ለምንያክል ጊዜ? ሀ. ሁል ጊዜ ለ. አልፎአልፎ

የዳታ ሰብሳቢ ስም _____ ያረጋገጠዉ _____

ፊርማ _____ ፊርማ _____

ቀን _____/ _____/ _____ ቀን _____/ ___/ ___

Annex IV: Laboratory procedures [60-63]

Thin smear preparation

1. Select and clean the preferable site to collect enough amount of blood from finger, heal, earlobe, even venous blood using a swab moistened with 70% alcohol , then air dry
2. Prink the finger with lancet, squeeze gently to obtain large amount of blood.
3. Using grease free frosted microscopic slide. Add a drop of blood on the slide 2mm far from rim of the slide, then spread the blood immediately without jerking to make thin smear with another slide.
4. Label the slide by using lead pencil on the edge of the slide or on the blood.
5. Fix with absolute methanol the thin part of the smear and left the thick one.
6. Air dry and stain finally examine under oil immersion.

Thick smear preparation

1. It is similar with thin smear Prink the finger with lancet, squeeze gently to obtain large amount of blood.
2. Using grease free frosted microscopic slide. Add a drop of blood on the slide 2mm far from rim of the slide, and then spread the blood immediately to make oval shape.
3. Allow to air dry.
4. Label the slide by using lead pencil on the edge of the slide or on the blood.
5. Stain with Giemsa and examine with oil immersion.

Giemsa Staining procedure:

1. Prepare 10% Giemsa working solution from Giemsa stock solution volume by volume that mean 1:10, it works for 24hrs.
2. Place the slide in the solution to Stain for 10 minutes.
3. Wash with distilled if it is accessible or buffered water.
4. Air dries the stained slide after wash the back of the slide.
5. Examine with 10x, 40x then 100x oil immersion objective to diagnose the plasmodium parasite and to see the morphology of the RBC morphology or to assess anemia.

6. Finally report the findings and interpret based on the parasite percentage.

Anthropometric measurement procedures

Materials:-MUAC measuring meter, weight balance (digital balance)

Height for children 24 months and older procedure

1. Place the measuring board on a hard flat surface against a wall, table, tree, staircase, etc. Make sure the board is not moving.
2. Ask the mother to remove the child's shoes and unbraid any hair that would interfere with the height measurement. Ask her to walk the child to the board and to kneel in front of the child. If a Microtoise measure is used, stand the child vertically in the middle of the platform.
3. Kneel on your right knee on the child's left side. This will give you maximum mobility.
4. Place the child's feet flat and together in the center of and against the back and base of the board/wall. Place your right hand just above the child's ankles on the shins, your left hand on the child's knees and push against the board/wall. Make sure the child's legs are straight and the heels and calves are against the board/wall. Tell the measurer when you have completed positioning the feet and legs.
5. Tell the child to look straight ahead at the mother who should stand in front of the child. Make sure the child's line of sight is level with the ground. Place your open left hand under the child's chin. Gradually close your hand. Do not cover the child's mouth or ears. Make sure the shoulders are level, the hands are at the child's side and the head, shoulder blades and buttocks are against the board/wall. With your right hand, lower the headpiece on top of the child's head. Make sure you push through child's hair.
6. Check the child's position. Repeat any steps as necessary.
7. When the child's position is correct, read and call out the measurement to the nearest 0.1 cm. Remove the headpiece from the child's head and your left hand from the child's chin.

8. Immediately record the measurement and show it to the measurer.
9. Check the recorded measurement on the questionnaire for accuracy and legibility.

Weight Using Salter-like Hanging Scale procedure

1. Hang the scale from a secure place like the ceiling beam. You may need a piece of rope to hang the scale at eye level. Ask the mother to undress the child as much as possible.
2. Attach a pair of the empty weighing pants to the hook of the scale and adjust the scale to zero, and then remove from the scale.
3. Have the mother hold the child. Put your arms through the leg holes of the pants. Grasp the child's feet and pull the legs through the leg holes. Make certain the strap of the pants is in front of the child.
4. Attach the strap of the pants to the hook of the scale. Do not carry the child by the strap only. Gently lower the child and allow the child to hang freely.
5. Stand behind and to one side of the measurer ready to record the measurement.
6. Check the child's position. Make sure the child is hanging freely and not touching anything.
7. Hold the scale and read the weight to the nearest 0.1 kg. Call out the measurement when the child is still and the scale needle is stationary. Even children, who are very active, which causes the needle to wobble greatly, will become still long enough to take a reading. Wait for the needle to stop moving.
8. Immediately record the measurement.
9. Records the measurement, gently lift the child by the body. Do not lift the child by the strap of the weighing pants. Release the strap from the hook of the scale.
10. Check the recorded measurement on the questionnaire for accuracy and legibility.

Child Mid-Upper Arm Circumference (MUAC) Procedure

Keep your work at eye level. Sit down when possible. Very young children can be held by their mother during this procedure. Ask the mother to remove clothing that may cover the child's left arm.

1. Calculate the midpoint of the child's left upper arm by first locating the tip of the child's shoulder with your finger tips. Bend the child's elbow to make a right angle. Place the tape at zero, which is indicated by two arrows, on the tip of the shoulder and pull the tape straight down past the tip of the elbow. Read the number at the tip of the elbow to the nearest centimeter. Divide this number by two to estimate the midpoint. As an alternative, bend the tape up to the middle length to estimate the midpoint. A piece of string can also be used for this purpose. Either you or an assistant can mark the midpoint with a pen on the arm.
2. Straighten the child's arm and wrap the tape around the arm at midpoint. Make sure the numbers are right side up. Make sure the tape is flat around the skin.
3. Inspect the tension of the tape on the child's arm. Make sure the tape has the proper tension and is not too tight or too loose. Repeat any steps as necessary.
4. When the tape is in the correct position on the arm with the correct tension, read and call out the measurement to the nearest 0.1cm.
5. Immediately record the measurement on the questionnaire and show it to the measurer.
6. While the assistant records the measurement, loosen the tape on the child's arm.
7. Check the recorded measurement on the questionnaire for accuracy and legibility. Instruct the assistant to erase and correct any errors.
8. Remove the tape from the child's arm.

Kato Katz technique [64]

Materials and reagents

Spatula, Applicator sticks, Template, Mesh (sieve), Glove Microscope, plastic sheet, Microscope slides, Forceps, Malachite green, Glycerol, cellophane, gauze, Petri dish, distilled water.

Kato Katz Procedure

1. Put approximately 2g stool on the plastic sheet or paper
2. Scrap the stool under the screen with spatula and stool will be sieved out of the screen
3. Place a template on microscopic slide
4. Fill the hole with finely sieved stool
5. Then clean extra stool on the template and remove the template from the measured stool carefully
6. Place a presoaked cellophane (50% glycerol malachite green over 24hrs)on the cylindrical stool
7. Apply a pressure with another slide to make equal distribution then remove by sliding gently
8. Incubate or left on the bench for water evaporation
9. Diagnose within 30minute for hook worm and continue for other intestinal helminths
10. Counting the ova of the parasite is important to determine parasite egg load
11. Finally record the finding on the laboratory data sheet

Hemoglobin determination procedure

1. A drop of blood filled the micro cuvette by touching the micro cuvette tip in the middle of the drop of blood until completely filled
2. Avoiding air bubble
3. Immediately after taking blood sample for malaria detection
4. The filled micro cuvette was put on the micro cuvette holder and push into hemacue instrument. The Hgb value was displayed in g/dl after approximately 30 seconds, and then was recorded.
5. The Hgb level lower than 11.5g/dl was considered as anemic and 12g/dl and above are considered as normal (Hgb normal value is 12-18 g/dl) [62].

Annex V: Data collection format

SECTION I: SOCIO-DEMOGRAPHIC CHARACTERISTICS

Identification no (ID NO) _____

1. Age (in figure)_____
2. Sex A. male B. female
3. Residence A. urban B. rural
4. Family educational level A. Illiterate B. Read and write C. Primary education (1-8) D. Secondary (9-10) E. College and above.
5. Guardian/ Mother Occupation A. Farmer B. Government employed C. G. unemployed D. Household E. Student F. Merchant
6. Is there any pond water Swampy area in your environment? A, Yes B, No

SECTION II: CLINICAL DATA

7. Weight (cm):____
8. Height (cm):____
9. Anemia A. Yes B. No
10. MUAC (cm):____

SECTION III: LABORATORY RESULT

11. Hgb (g/dl) _____
12. Malaria A. Yes B. No

12.1 *p.falciparum* = _____

12.2 p.vivax = _____

12.3 Mixed= _____

13. *A.lumbricoides* A. Yes B. No = egg load per gram (ELPG) _____

14. H.worm A. Yes B. No = egg load per gram (ELPG) _____

15. *S.mansoni* A. Yes B. No = egg load per gram (ELPG) _____

16. *T.species* A. Yes B. No = egg load per gram (ELPG) _____

17. *H.nana* A. Yes B. No = egg load per gram (ELPG) _____

18. *T.trichuria* A. Yes B. No = egg load per gram (ELPG) _____

Declaration

I, the undersigned, declare that this thesis is my own work and that all sources of material used for the thesis have been duly acknowledged.

Name of the student	Signature
Habtie Tesfa	_____

Submitted to: Department of Medical Parasitology School of Biomedical and Laboratory Sciences CMHS, University of Gondar.

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